Study of Nucleic Acid Binding of Aloe- emodin and Emodin by Spectroscopic Techniques

A SYNOPSIS
Submitted for the Award of Degree of
Doctor of Philosophy

By

Monika Yadav

Under the Supervision of

Prof. Surat Kumar
(Supervisior)

Prof. Sahab Dass
Head,
Department of Chemistry

Prof. Ravinder Kumar
Dean,
Faculty of Science

Dayalbagh Educational Institute, Dayalbagh, Agra
March, 2015

INTRODUCTION

Cancer or tumour is a complex and dynamic disease, involving a variety of changes in the gene expression and structure. It involves disordered cell replication that is death and disorganization of organ structure. These cancer cells are malignant in nature. They do not die until host dies. Secondly they undergo metastasis i.e. transfer of cancer cells from one organ of the body to another which is not directly connected to it. It is considered as a major cause of mortality worldwide, and it strikes more than one-third of the world's population and is the cause of more than 20% of all deaths [1].

Tumours are either 'benign' or 'malignant'. Benign tumours are generally slow-growing expaning masses that compress rather than invade surrounding tissue. Malignant tumours are usually rapidly growing, invading surrounding tissue and most significantly, colonizing distant organs [2].

To cure this disease, several therapies have been employed over the years. In combination with surgery and radiotherapy, chemotherapy has been effectively employed for the treatment of cancer. The aim of most chemotherapeutic agents or anticancer drugs currently in clinical use is to kill malignant tumour cells by inhibiting some of the mechanisms implied in cellular division. Cancer chemotherapy is a very difficult task. One of its main associated problems is the non-specific toxicity of most anticancer drugs due to their distribution throughout the body. Cancerous cells which are initially suppressed by a specific drug may develop a resistance to that drug. For this reason, cancer chemotherapy may consist of using several drugs in combination for varying lengths of time [3].

In order to find better therapies, many scientists and researchers have discovered that synthetic anticancer medicines were a highly effective treatment to eliminate the cancer cells. However, they become less effective in treating recurring cancer cells, and many of them damage the normal cells

and have mutation heredity toxicity in varying degrees [4]. Correspondingly, the toxicity of herbal anticancer drugs is not as pronounced and herbal anticancer drugs have been developed to take a more holistic approach. Furthermore, the toxicity of the herbal drug is very low and most of them have no side effect. The exploitation of the natural source makes the difference in the field of the medicine.

The search for anti-cancer agents from plant sources started in earnest in the 1950s with the discovery and development of the Vinca alkaloids, Vinblastine and Vincristine, and the isolation of the cytotoxic Podophyllotoxins. As a result, National Cancer Institute (NCI) in the United States initiated an extensive plant collection program in 1960, focused mainly in temperate regions. This led to the discovery of many novel chemotypes showing a range of cytotoxic activities [5], including the taxanes and camptothecins.

Different phytochemicals derived from plants, including anthraquinones, are considered to possess anticancer potential . The anthraquinones are bioactive compounds, which are present as glycosides in various plants. Some anthraquinones and their derivatives, such as Emodin, Aloe-emodin, Danthron, Chrysophanol, Physcion, and Rhein, demonstrate anticancer properties [6].

Aloe-emodin (1,8-dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione; Figure 1**)** is a naturally occurring anthraquinone derivative present in the leaves and roots of a number of plants, such as aloe and senna. Aloe-Emodin is able to inhibit cell growth in several tumor cells, including human lung carcinoma [7], hepatoma [8] and leukemia cell lines [9]. Aloe-emodin showed a high specificity for neuroectodermal tumor cells [10 and 11].

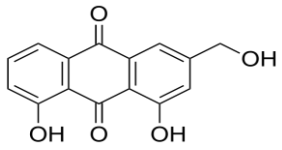

Figure 1: Structure of Aloe Emodin

Emodin (1,3,8-trihydroxy-6-methylanthraquinone; Figure 2) an antraquinone and one of the active ingredients, isolated from the rhizome of rhubarb, a plant of traditional Chienese medicine [12]. This herb is commonly employed as a purgative crude herb in traditional herbal therapy and has specific *in vitro* and *in vivo* anti-tumor activity. The known pharmacological effects of Emodin include antitumor, anti-microbial, immunosuppressive and anti-inflammatory activities [13]. It has anti-tumor activity in human chronic myelocytic leukemia [14].

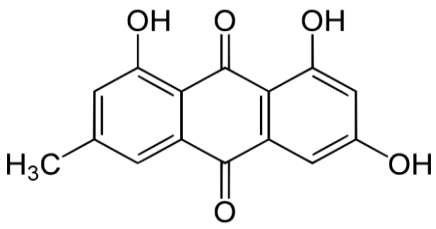

Figure 2: Structure of Emodin

DRUG: NUCLEICIC ACID INTERACTIONS

Nucleic acids are the major target for drug interaction as it is the origin point of most important cellular processes including replication, transcription and translation. These processes are vital for cell growth and division. DNA starts transcribing or replicating only when it receives a signal, which is often in the form of a regulatory amino acid binding to a particular region of the DNA. Thus, if the binding specificity and strength of this regulatory protein can be mimicked by a small molecule, then nucleic acid functions can be artificially modulated, inhibited or activated by binding this molecule instead of the protein. Thus, synthetic/natural small molecules can act as drugs when activation or inhibition of nucleic acid function culminates into the cure or control of a disease. These synthetic or naturally occurring small molecules have clinical applications and many of them are under clinical trial.

Over the last two decades enormous advances and efforts have been made to study the ways in which ligands i.e.drug molecules bind with nucleic acids. Both the covalent and non-covalent modes of binding are observed for the drug-nucleic acid complexation. There are three main types of drug-nucleic acid binding modes which are used to classify the drug-DNA interaction: intercalation, minor groove binding, and major groove binding [15]. Intercalators usually have planar aromatic ring systems that are inserted between two neighbouring bases into the DNA helix. This leads to increased distance between the adjacent bases and local unwinding of the double helical structure of DNA. Intercalating drugs induce structural perturbations in the Nucleic acid structure to accommodate the drug binding, such as the unwinding of the double helix, in turn lengthening of the DNA [16, 17 and 18]. Often, the stacking of the bases in the rest of the helix is very little influenced. The intercalation is stabilized by stacking interactions, i.e. π-π interactions between the aromatic rings. Typical intercalating drugs are ethidium bromide (Figure 3), Ellipticine, Epirubicin etc.

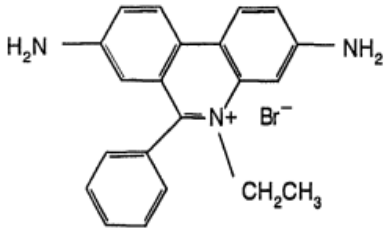

Figure 3: Structure of Ethidium Bromide

A second, fundamental concept that emerged from the examination of naturally occurring nucleic acid binding molecules is bisintercalation. A new classic series of natural products have been identified since the seminal studies on Echinomycin [19] and Triostin-A [20] that have been shown to bind by bisintercalation. Of these, the binding of Sandramycin is perhaps the most straight-forward to appreciate [21].

Groove binding small molecules are more recent additions to the field [16]. Minor groove binders are typically long elongated structures with a curvature that fits the curvature of the DNA minor groove. The ligand is fitted between the narrow walls of the minor groove and stabilized via H-bonds and van der Waals interactions. The DNA minor groove also has a certain flexibility to accommodate for ligands that do not have a perfect fit. Drugs in this category include Netropsin, Distamycin and Hoechst-33258 etc.

Major groove binders utilize the numerous possibilities for specific H-bond donors and acceptors on the nucleic bases providing the basis for both complex stabilization and sequence specificity [22]. Surface binders interact electrostatically and/or via H-bonds to the phosphate backbone on DNA. The binding is often non-specific and is difficult to observe directly. Typically only indirect binding is detectable, for example as a change in the backbone configuration.

LITERATURE REVIEW

The interaction of various anticancer drugs with different DNAs and RNAs has been reported by various research groups, using spectroscopic, electrochemical and molecular modeling techniques. The interaction of anti-cancer herbal drug Berberine with ds-DNA (fish test DNA) immobilized on Glassy Carbon Electrode was studied using CV, DPV and UV spectroscopy. The studies were conducted in Britton–Robinson buffer solution (0.05 M, pH 5.72) at T=298 K (25 °C). The presence of DNA results in a decrease of the current and a negative shift of the electrode potentials from the DPV curves of Berberine, indicating the dominance of electrostatic interactions. The binding constant was also calculated [23].

Wang et al. investigated the interaction of emodin with DNA by electrochemistry and UV spectra. The results indicated that the interaction between DNA and emodin is that emodin intercalates into the double helix of DNA. There is no electrostatic interaction between emodin and DNA. Emodin after binding with DNA formed a complex of DNA–2Emodin with (combining constant) $\beta = 6.61\times10^{9}$ via intercalation. Therefore, emodin can prevent cancerous cells from dividing and producing more cancer cells by inhibiting DNA replication and simultaneously exhibits the toxic effect to a certain extent. Furthermore, emodin can be used to determine the DNA concentration as a new electrochemical probe [24].

The interaction of the natural plant alkaloid and anticancer agent Sanguinarine with t-RNA^{phe} has been investigated by spectroscopic and calorimetric techniques. Sanguinarine iminium binds cooperatively to t-RNA^{phe}. The binding affinity of the Sanguinarine iminium to t-RNA^{phe} obtained from isothermal titration calorimetry and measured in the order of 10^{5} M^{-1}. The binding was driven largely by negative enthalpy and a smaller but favourable positive entropy change and was dependent on the [Na^{+}] concentration, but had a larger non-electrostatic contribution to the Gibbs energy. A small heat capacity value and the enthalpy–entropy compensation in the energetics of the

interaction, characterized the binding of the iminium form to t-RNA^{phe}. This study confirmed that the t-RNA^{phe} binding moiety is the iminium form of Sanguinarine [25].

The interaction of two natural protoberberine plant alkaloids viz. berberine and palmatine with t-RNA^{phe} was studied using various biophysical techniques and molecular modeling and the data were compared with the binding of the classical DNA intercalator, ethidium bromide. The cooperative binding of both the alkaloids and ethidium bromide to t-RNA was revealed from absorbance and fluorescence studies. Fluorescence quenching studies advanced a conclusion that while berberine and palmatine are partially intercalated, ethidium is fully intercalated on the t-RNA molecule. The binding of the alkaloids as well as ethidium stabilized the t-RNA melting, and the binding constant evaluated from the averaged optical melting temperature data was in agreement with fluorescence spectral study of the t-RNA binding data. Differential scanning calorimetry revealed that the t-RNA melting showed three close transitions that were affected on binding of these small molecules. Molecular docking calculations performed showed the preferred regions of binding of these small molecules on the t-RNA [26].

Bourassa et al. showed that tamoxifen and its metabolites bind t-RNA with overall binding constants of $K_{tam–t\text{-}RNA}$= 5.2 (±0.6) × 10^4 M^{-1}, $K_{4\text{-}hydroxytam–t\text{-}RNA}$= 6.5 (±0.5) × 10^4 M^{-1} and $K_{endox–t\text{-}RNA}$= 1.3 (±0.2) × 10^4 M^{-1}. Docking showed the participation of several nucleobases in drug–t-RNA complexes with the free binding energy of −4.31(tamoxifen), −4.45 (4-hydroxtamoxifen) and −4.38 kcal/mol (endoxifen). Drug binding did not alter t-RNA conformation from A-family structure, while biopolymer aggregation occurred at high drug concentration [27]. The tamoxifen and its metabolites bind in the order of:

4-hydroxytamoxifen > tamoxifen > endoxifen

Kim et al. studied Interaction and binding geometries of complexes of Methyl green with poly(dA-dT)$_2$, poIy(dA) . poly(dT), and triplex poly(dA) . 2poly (dT) complexes by linear dichroism. For both of the complexes with double helical DNAs, the z-symmetry axis of Methyl green is found to be approximately parallel to the DNA bases; while the x symmetry axis lies at 40-44^0 relative to the local DNA helix axis, in agreement with a major groove binding mode. However, in contrast to DNA minor-groove binders (such as DAPI and Hoechst-33258) Methyl green is found to be excluded from binding to the DNA triple helix, i.e. poly(dA) . 2 poly(dT) in which the major groove is filled by the third strand. While most of the so far studied groove-binding dyes bind in the minor groove of DNA, Methyl green thus appears to be an exception [28].

Pecere et al. studied the anticancer activity of aloe emodin against Neuroectodermal tumors and reported that aloe-emodin (AE), a hydroxyanthraquinone present in Aloe vera leaves, has a specific *in vitro* and *in vivo* anti-neuroectodermal tumor activity. The growth of human neuroectodermal tumors is inhibited in mice with severe combined immunodeficiency without any appreciable toxic effects on the animals. The compound does neither inhibit the proliferation of normal fibroblasts nor that of hemopoietic progenitor cells. The cytotoxicity mechanism consists of the induction of apoptosis, whereas the selectivity against neuroectodermal tumor cells is found on a specific energy-dependent pathway of drug incorporation. Taking into account its unique cytotoxicity profile and the mode of action, AE might represent a conceptually new lead antitumor drug [10].

Hong-yan et al. examined the interaction between Aloe-emodin and DNA by Cyclic Voltammetry and observed that the interaction was mainly of an electrostatic one in phosphate buffer solution at pH 4.4,whereas intercalative binding played a dominant role at pH 6.5 and 7.4 solutions. The results of the UV-Vis spectroscopic study further confirmed them. With the kinetic parameters, such as charge transfer coefficient α and standard electron transfer rate constant K_s in the absence and

presence of DNA for the calculation, the calculated results showed that an electrochemical active supramolecular complex was formed between AE and DNA [29].

Saito et al. examined the molecular interactions of emodin with DNA in aqueous solution at physiological pH and observed the binding constant for emodin–DNA complex with K= 5.59 x 10^3 M^{-1} and mode of binding was intercalation and external binding FTIR results suggested that emodin interaction occurs preferably via adenine and thymine base pairs and also weakly with the phosphate backbone of the DNA double helix [30].

Bi et al. studied the interaction of emodin and DNA in the presence of ethidium bromide (EB) by spectroscopic method; the binding constants of emodin with DNA in the presence of EB are 6.02 × 10^4, 9.20 × 10^4 and 1.17 × 10^5 L mol^{-1} at 20, 35 and 50°C respectively. FT-IR spectrum further suggests that both the phosphate groups and the bases of DNA react with emodin. The reaction of DNA with emodin in the presence of EB is affected by ionic strength and temperature [31].

HYPOTHESIS

The study on the interaction of drug and Nucleic acids plays a key role in pharmacology. It is of great significance for designing and synthesizing the new drugs targeted to Nucleic acids. Their effectiveness depends on the mode and affinity of the binding. Anthraquinones is a widely distributed compound in nature, which is the effective component of lots of natural herbs. They possess antibacterial, diuretic, antiviral and antitumor effects. Since, the interaction of Aloe-Emodin and Emodin with CT-DNA has been studied by several techniques like UV-Vis, FTIR, Fluorescence spectroscopy, Raman spectroscopy, Circular- Dichroism, Cyclic voltammetry , etc. but the detailed structure, specificity and mode of binding of these drugs and Nucleic acids complex have not been studied yet. The aim of this study was to examine the specificity, detailed structure and molecular interactions of Aloe-emodin and emodin with Nucleic Acid by spectrophotometric methods, electrochemical methods and spectroscopic techniques.

OBJECTIVES

1. To study the interaction of anticancer anthraquinone with different designed DNA oligomers by various spectrophotometric techniques.
2. To investigate the interaction of proposed anthraquionone with RNAs by spectrophotometric techniques.
3. To study the interaction of anticancer anthraquinone with different designed DNA oligomers by various electrochemical techniques.
4. To investigate the interaction of proposed anthraquionone with RNAs by electrochemical techniques.
5. To evaluate the thermodynamic parameters of complex of propose drugs with different DNAs and RNAs.
6. The comprehensive structural study of a proposed Drug- DNA complex by NMR techniques.

MATERIALS AND METHODS

Sample Preparation

Drugs emodine and aloe-emodine will be purchased from Sigma-Aldrich Chemical Co. DNA oligomers are self-complementary and form a double helical structure with the help of H-bonds. Following DNA oligomers and natural RNA are proposed to carrying out our research work:

DNA Oligomers

1. DNA-1: 5'-d(GATGGCCATC)$_2$
2. DNA-2: 5'-d(GATCCGGATC)$_2$
3. DNA-3: 5'-d(GGCAATTGCC)$_2$
4. DNA-4: 5'-d(GGCTTAAGCC)$_2$

RNA

1. Natural yeast-RNA
2. Natural yeast- transfer RNA

The proposed anticancer drugs to carry out our studies are

1. Aloe-emodin
2. Emodin

The stock solution of nucleic acids will be prepared by dissolving appropriate amount of nucleic acids in doubly distilled water and stored at 4 ºC for use. The concentration of DNA oligomers would be determined spectrophotometrically by using the specific molar extinction coefficients. The ratio of the absorbance at 260 and 280 nm would be checked to be ~1.89, indicating that the nucleic acids are sufficiently free from proteins.

Spectrophotometric Techniques

All Spectrophotometric measurements will be recorded at a spectrophotometer (UV or Fluorescence) where a fixed concentration of drugs will be titrated with increasing concentration of nucleic acids in a buffer which is fluorescent free with a quartz quvette of 1cm path length at different pH. Binding constant would be calculated by double reciprocal plot which is 1/[Nucleic Acids] vs. 1/[difference in absorbance or emission] and this gives the straight line with an intercept and slope.

$$\frac{1}{[DNA]} = \frac{1}{K} + \frac{1}{[Difference\ of\ absorption\ or\ emission]}$$

Study of Thermodynamic Parameters

The thermodynamic parameters of Drugs - Nucleic Acids binding will be calculated by carrying out fluorescence titration with fixed concentration of drugs and increasing aliquots of Nucleic Acid concentration at different temperature. Thermodynamic parameters would be estimated by the analysis of ln K vs. 1/T plot (van't Hoff plot). The gradient of this straight line of ln K versus 1/T is equal to -ΔH/R which indicates the value of ΔH. ΔG and ΔS can be calculated from the following fundamental thermodynamic relationships:

$$\ln K = \frac{1}{T} - \frac{\triangle H}{R}$$

$$\triangle G = -RT \ln K$$

$$\triangle S = \frac{\Delta H - \Delta G}{T}$$

Electrochemical Studies

The electrochemical analysis of the drug-Nucleic acid interactions is mainly based on the differences in the redox behavior of the nucleic acid-binding molecules in the absence and presence of nucleic acid, including the shifts of the formal potential of the redox couple and the decrease of the peak current resulting from the dramatic drop in the diffusion coefficient after association with Nucleic acids [32].

The electrochemical studies will be furnished by DNA biosensor fabrication technique by using suitable working electrode and different electrochemical methods i.e., cyclic voltammetry, differential Pulse Voltammetry etc. [23, 24, 33].

NMR Studies

After the optimization of experimental conditions and evaluation of binding parameters for both the proposed drugs with DNA, it is required to determine the elaborated structural features of one of the proposed drug-DNA complex by a precise technique i.e. NMR. 1D ^{1}H-NMR and 2D ^{1}H-NOESY experiments would be conducted on the proposed drug–DNA complex as reported [34, 35].

REFERENCES

1. Foye, W. O., Lemke, T. L., Williams, D. A., Roche, V. F. and Zito, S. W. (2008), Foye's principles of medicinal chemistry, Philadelphia: Lippincott Williams & Wilkins, (6th ed.), 1147-1148.
2. Alison, M. R. (2001), Cancer, Imperial College School of Medicine, London, UK, Published online Encyclopedia of Life Sciences.
3. Chorawala, M. R., Oza, P. M. and Shah, G. B. (2012), Mechanisms of Anticancer Drugs Resistance: An Overview, *Int. J. Pharma. Sci.Drug Res.*, **1**, 1-9.
4. Fouzia, P., Rumana, Q., Ansari, L. F., Kalsoom, S. and Ahmed, S. (2011), Investigations of drug–DNA interactions using molecular docking, cyclic voltammetry and UV–Vis spectroscopy, *J. Mol. Struct.*, **1004,** 67-73.
5. Cassady, J. M. and Douros, J. D. (1980), Anticancer agents based on natural product models, *New York: Academic Press*.
6. Yordanova, A. and Koprinarova, M. (2014), Is aloe-emodin a novel anticancer drug, *Trakia J. Sci.*, **12**, 92-95.
7. Lee, H. Z., Hsu, S. L., Liu, M. C. and Wu, C. H. (2001), Effects and mechanisms of aloe-emodin on cell death in human lung squamous cell carcinoma. *Eur. J. Pharmaco*l., **431**, 287-295.
8. Yeh, F. T., Wu, C. H. and Lee, H. Z. (2003), signaling pathway for aloe-emodin-induced apoptosis in human H460 lung nonsmall carcinoma cell, *Int. J. Cancer*, **106**, 26-33.
9. Chen, H. C., Hsieh, W. T., Chang, W. C. and Chung, J. G. (2004), Aloe-emodin induced in vitro G2/M arrest of cell cycle in human promyelocytic leukemia HL-60 cells. *Food Chem. Toxicol.*, **42**, 1251-1257.
10. Pecere, T., Gazzola, M. V., Mucignat, C., Parolin, C.,Vecchia, F. D., Cavaggioni, A., Basso, G., Diaspro, A., Salvato, B., Carli, M. and Palu, G. (2000), Aloe-emodin is a new

type of anticancer agent with selective activity against neuroectodermal tumors. *Cancer Res.*, **60**, 2800-2804.

11. Wang, J. Z., He, J. X. and Jiang, C. Q. (2001), The interaction of serum albumin with aloe-emodin. *Chin. J. Anal. Chem,*, **29**, 782-784.
12. Bi, S. Y., Song, D. Q., Kan, Y. H, Xu, D., Tian, Y., Zhou, X. and Zhang, H. (2005), Spectroscopic characterization of effective components anthraquinones in Chinese medicinal herbs binding with serum albumins, *Spectrochim. Acta A*, **62**, 203-212.
13. Chukwujekwu, J. C., Coombes, P. H., Mulholland, D. A. and Staden, J. V. (2006), Emodin, an antibacterial anthraquinone from the roots of Cassia occidentalis, *South African J. Bot.*, **72**, 295-297.
14. Srinivas, G., Babykutty, S., Sathiadevan, P. P. and Srinivas, P. (2007), Molecular mechanism of emodin action: transition from laxative ingredient to an antitumor agent, *Med. Res. Rev.*, **27**, 591- 608.
15. Johnson, D. S. and Boger, D. L. (1996), DNA binding agents. In Comprehensive Supramolecular Chemistry, Y. *Murakami, ed. (Oxford: lsevier)*, 73-176.
16. Searle, M. S., Hall, J. G., Denny, W. A. and Wakelin, L. P. G. (1988), NMR Studies of the Interaction of the Antibiotic Nogalamycin with the Hexadeoxyribonucleotide Duplex d(5'-GCATGC), *Biochem*., **27**, 4340-4349.
17. Searle, M. S., Bicknell, W., Wakelin, L. P. G. and Denny, W. A. (1991), Anthracycline antibiotic arugomycin binds in both grooves of the DNA helix simultaneously: an NMR and molecular modelling study, *Nucleic Acids Res.*, **19**, 2897-2906.
18. Sun, D. Y., Hansen, M., Clement, J. J. and Hurley, L. H. (1993), Structure of the Altromycin B (N7-Guanine)-DNA Adduct. A Proposed Prototypic DNA Adduct Structure for the Pluramycin Antitumor Antibiotics, *Biochem.*, **32**, 8068-8074.
19. Waring, M. J. and Wakelin, L. P. G., (1974), Echinomycin A bi functional intercalating antibiotic, *Nature*, **252**, 653-657.

20. Shoji, J. I. and Katagiri, K. (1961), Studies on quinoxaline anti biotics. II. New antibiotics, triostins A, B and C, *J. Antibiot. (Tokyo)*, **14**, 335-339.

21. Boger, D. L., and Saionz, K. W. (1999), DNA binding properties of key sandramycin analogues: systematic examination of the intercalation chromophore. *Bioorg. Med. Chem.*, **7**, 315-321.

22. Searle, M. S. (1993), NMR studies of Drug-DNA interaction, *Prog. Nucl. Magn. Reson. Spectrosc*., **25**, 403-480.

23. Tian, X., Song, Y., Dong, H. and Ye, B. (2008), Interaction of Anticancer Herbal Drug Berberine with DNA Immobilized on the Glassy Carbon Electrode, *Bioelectrochem.*, 73, 18-22.

24. Wang, L., Lin, L. and Ye, B. (2006), Electrochemical studies of the interaction of the anticancer herbal drug emodin with DNA, *J. Pharmac. Biomed. Anal.*, **42**, 625-629.

25. Chowdhury, S. R., Islamzand, M. M. and Gopinath, S. K. (2010), Binding of the anticancer alkaloid sanguinarine to double stranded RNAs: Insights into the structural and energetics aspects, *Mol. Bio. Syst.*, **6**, 1265-1276.

26. Islam, M. M., Pandya, P., Chowdhury, S. R., Kumar, S. and Kumar, G. S. (2008), Binding of DNA-binding alkaloids berberine and palmatine to tRNA and comparison to ethidium: Spectroscopic and molecular modeling studies, *J. Mol. Struct.*, **891**, 498-507.

27. Bourassa, P., Thomas, T. J., Bariyanga, J. and Tajmir-Riahi, H. A. (2015), Breast anticancer drug tamoxifen and its metabolites bind tRNA at multiple sites International, *Int. J. Biol. Macromol.*, **72**, 692-698.

28. Sinha, R. and Kumar, G. S. (2009), Interaction of Isoquinoline Alkaloids with an RNA Triplex: Structural and Thermodynamic Studies of Berberine, Palmatine, and Coralyne Binding to Poly(U).Poly(A)*Poly(U), *J. Phys. Chem. B*, **113**, 13410-13420.

29. Hong-yan, W., Ya-feng, Z., Jing-hua, C., Ping, W. U., Yan-jie, Z. and Xin-hua, L. (2007), Studies on the Interaction between Aloe-emodin and DNA with UV-Vis Spectrometry and Cyclic Voltammetry, *J. Instru. Anal.*, **26**, 59-61.

30. Saito, S. T., Silva, G., Pungartnik, C. and Brendel, M. (2012), Study of DNA–emodin interaction by FTIR and UV–vis spectroscopy, *J. Photochem. Photobio. B: Biol.*, **111**, 59-63.

31. Bi, S., Zhangc, H., Qiao, C., Sun, Y. and Liu, C. (2008), Studies of interaction of emodin and DNA in the presence of ethidium bromide by spectroscopic method, *Spectrochem. Acta Part A*, **69**, 123-129.

32. Ravera, M., Bagni, G., Mascini, M. and Osella, D. (2007), DNA-Metallodrugs Interactions Signaled by Electrochemical Biosensors: An Overview, *Bioinorg. Chem. App.*, **2007**, 1-10.

33. Sun, W., Zhao, N., Jia, K. (2007), Determination of Yeast RNA Using Safranine T with Linear Sweep Voltammetry, *Acta Chim. Slov.*, **54**, 778-783.

34. Kumar, S., Jaseja, M., Zimmermann, J., Bathini, Y., Pon, R. T., Sapse, A. M. and Lown, J. W. (1990), Molecular Recognition and Binding of a GC-Site Avoiding Thiazole-Lexitropsin to the Decadeoxyribonucleotide d-$(CGCAAT\text{-}TGCG)_2$: A ^{1}H-NMR Evidence for Thiazole Intercalation, *J. Biomol. Struc. Dyn.*, **8**, 99-121.

35. Kumar, S., Bathini, Y., Zimmermann, J., Pon, R. T. and Lown, J. W. (1990), Sequence Specific Molecular Recognition and Binding of a GC Recognizing Hoechst 33258 Analogue to the Deca deoxyribonucleotide d-$(CATGGCCA\text{-}TG)_2$: Structural and Dynamic Aspects Deduced from ^{1}H-NMR Studies, *J. Biomol. Struc. Dyn.*, **8**, 331-357.